REMARKS

The foregoing amendments amend the specification to reflect the 371 status. In addition, minor amendments have been effected to the specification.

On page 5, line 11, the compound "Taxotere" is deleted because Taxotere is the trade name for docetaxel which is described on the same line. On page 7, line 10, a minor typographical error has been corrected.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached pages are captioned "**Version with markings to show changes made**".

Favorable Action on the merits is solicited.

Respectfully submitted,

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DESCRIPTION

ANTITUMOR AGENT

This application is a 371 of PCT/JP00/00002 filed January 4, 2000.

Technical Field

This invention relates to the use of a combination of a microtubule-interfering agent and an ERK-MAP kinase cascade inhibitor as an antitumor agent.

Background Art

Microtubules are tubular protein fibers having a diameter of about 25 nm and occurring extensively in the cells of eucaryotes such as animals, plants and fungi, and they have a wide diversity of functions covering the formation of mitotic apparatus, the development and maintenance of cellular forms, flagellar and ciliary movements, the disposition of intracellular organellas, material transport, hormone secretion, the fluidity of cell membrane, and the like. Especially in nerve cells, microtubules exist as the principal constituent molecules of axons and dendrites, and contribute to material transport by acting as rails for motor proteins. These microtubules are formed by the regular polymerization of the αβ heterodimer of tubulin, and repeatedly show an increase or decrease by polymerization or depolymerization with the progress of the cell cycle. Moreover, the polymerization/depolymerization of these microtubules is also controlled by microtubule-associated proteins (MAPs, τ protein). This control mechanism is primarily based on protein phosphorylation enzymes (kinases) and protein dephosphorylation enzymes (phosphatases) which have those microtubule-associated proteins as substrates. For these reasons, compounds acting on the microtubule system (i.e., microtubule-interfering agents) exhibit various biological activities such as the inhibition of cell divisions, and are hence ex-

and the ERK-MAP kinase cascade inhibitor are administered, either simultaneously or separately with a predetermined interval of time, in order to treat tumors.

According to the present invention, there is also provided an antitumor agent which contains, as an active ingredient, a microtubule-interfering agent for use in combination with an ERK-MAP kinase cascade inhibitor.

According to the present invention, there is also provided an agent for potentiating the antitumor effect of a microtubule-interfering agent which contains an ERK-MAP kinase cascade inhibitor as an active ingredient.

According to the present invention, there is also provided a method for the treatment of a tumor which comprises administering a microtubule-interfering agent and an ERK-MAP kinase cascade inhibitor to a patient, either simultaneously or separately with a predetermined interval of time.

Moreover, according to the present invention, there is provided a method for the use of an ERK-MAP kinase cascade inhibitor for the purpose of potentiating the antitumor effect of a microtubule-interfering agent.

Furthermore, according to the present invention, there is provided a pharmaceutical product comprising a microtubule-interfering agent and/or an ERK-MAP kinase cascade inhibitor, the pharmaceutical product being characterized by having, on or within the packaging material thereof, an indication or document showing the instruction that the microtubule-interfering agent and the ERK-MAP kinase cascade inhibitor should be used in combination.

The term "microtubule-interfering agent" as used herein means any drug that acts on the microtubule system and thereby exhibits an antitumor effect. According to their site of action, they are classified into compounds binding to β-tubulin and compounds

binding to microtubule-associated proteins. Moreover, the compounds binding to β-tubulin are classified into compounds inhibiting the polymerization of tubulin by binding to β-tubulin and compounds promoting the abnormal polymerization of tubulin to the contrary. Specifically, the compounds inhibiting the polymerization of tubulin include, for example, dolastatin 10 and compounds analogous thereto, vincristine and compounds analogous thereto, maytansine (THE MERCK INDEX 12th EDITION No. 5800), rhizoxin, phomopsin, ustiloxin and combrestatin. The compounds promoting the polymerization of tubulin include, for example, paclitaxel (THE MERCK INDEX 12th EDITION No. 7117), docetaxel~~, Taxotere~~ and taxuspine.

On the other hand, the compounds binding to microtubule-associated proteins include, for example, griseofulvin (THE MERCK INDEX 12th EDITION No. 4571) and estramustine (THE MERCK INDEX 12th EDITION No. 3749).

The compound which can be preferably used as microtubule-interfering agents in the present invention are compounds inhibiting the polymerization of tubulin. Among them, dolastatin 10 and compounds analogous thereto, and vincristine and compounds analogous thereto are especially preferred.

Specific examples of these dolastatin 10 and compounds analogous thereto include, for example, the compounds described in references such as Japanese Patent Laid-Open No. 167278/'90, PCT International Publication WO93/03054 Pamphlet, PCT International Publication WO95/09864 Pamphlet, PCT International Publication WO96/33212 Pamphlet, Japanese Patent Laid-Open No. 293795/'94, Japanese Patent Laid-Open No. 70173/'95, Japanese Patent Laid-Open No. 59693/'96, Japanese Patent Laid-Open No. 81493/'96, Japanese Patent Laid-Open No. 119990/'96, Japanese Patent Laid-Open No. 188594/'96, Japanese Patent Laid-Open No. 77791/'97, Published Japanese Translation of PCT International Publication No. 506580/'95,

tively formed, and the compounds which inhibit the action of activated MAP kinase.

Specific examples of these ERK-MAP kinase cascade inhibitors include, for example, 2-(2-amino-3-methoxyphenyl)-4H-chromen-4-one (hereinafter referred to as "PD98059"; Proc. Natl. Acad. Sci., Vol. 92, pp. 7686-7689, 1995), 1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene (hereinafter referred to as "U1026"; J. Biol. Chem., Vol. 273, pp. 18623-18632, 1998), and 2-(2-chloro-4-iodophenylamino)-N-cyclopropylmethoxy-3,4-difluorobenzamide (hereinafter referred to as "~~PD1843522~~ PD184352"; Nature Medicine, Vol. 5, pp. 810-816, 1999).

If necessary, the microtubule-interfering agent or ERK-MAP kinase cascade inhibitor used in the present invention may be reacted with an inorganic or organic acid or an inorganic or organic base to form a pharmaceutically acceptable salt. The inorganic acids which can be used to form a salt include, for example, hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid and phosphoric acid, and the organic acids which can be used include, for example, acetic acid, propionic acid, oxalic acid, malonic acid, succinic acid, lactic acid, malic acid, tartaric acid, citric acid, benzoic acid and methanesulfonic acid. On the other hand, the inorganic bases which can be used to form a salt include, for example, sodium hydroxide, ammonium hydroxide, calcium carbonate, sodium hydrogen carbonate and calcium hydroxide, and the organic bases which can be used include, for example, methylamine, diethylamine, cyclohexylamine, ethanolamine and morpholine.

The above-described microtubule-interfering agent and ERK-MAP kinase cascade inhibitor may be used in the form of a pharmaceutical preparation having any of dosage forms including solid forms (e.g., tablets, hard capsules, soft capsules, granules, powders, fine subtilaes, pills and troches), semisolid forms (e.g.,